

No.	Key	Function
1	Light Source	Provides photo excitation
2	Planar Wave Guide (PWG)	Sample substrate & carrier for emission light
3	Photo Detector	Detects light emitted from sample
4	Emission Filter	Filter that allows only emission wave length to pass
5	Optics	Collimates light emitted from lamp
6	Excitation Filter	Filter that allows only excitation wave length to pass
7	Lamp	Produces light
8	Excitation Rays	
9	Emission Rays	
10	Probe Surface	Target Hybridization for later detection

Figure 1

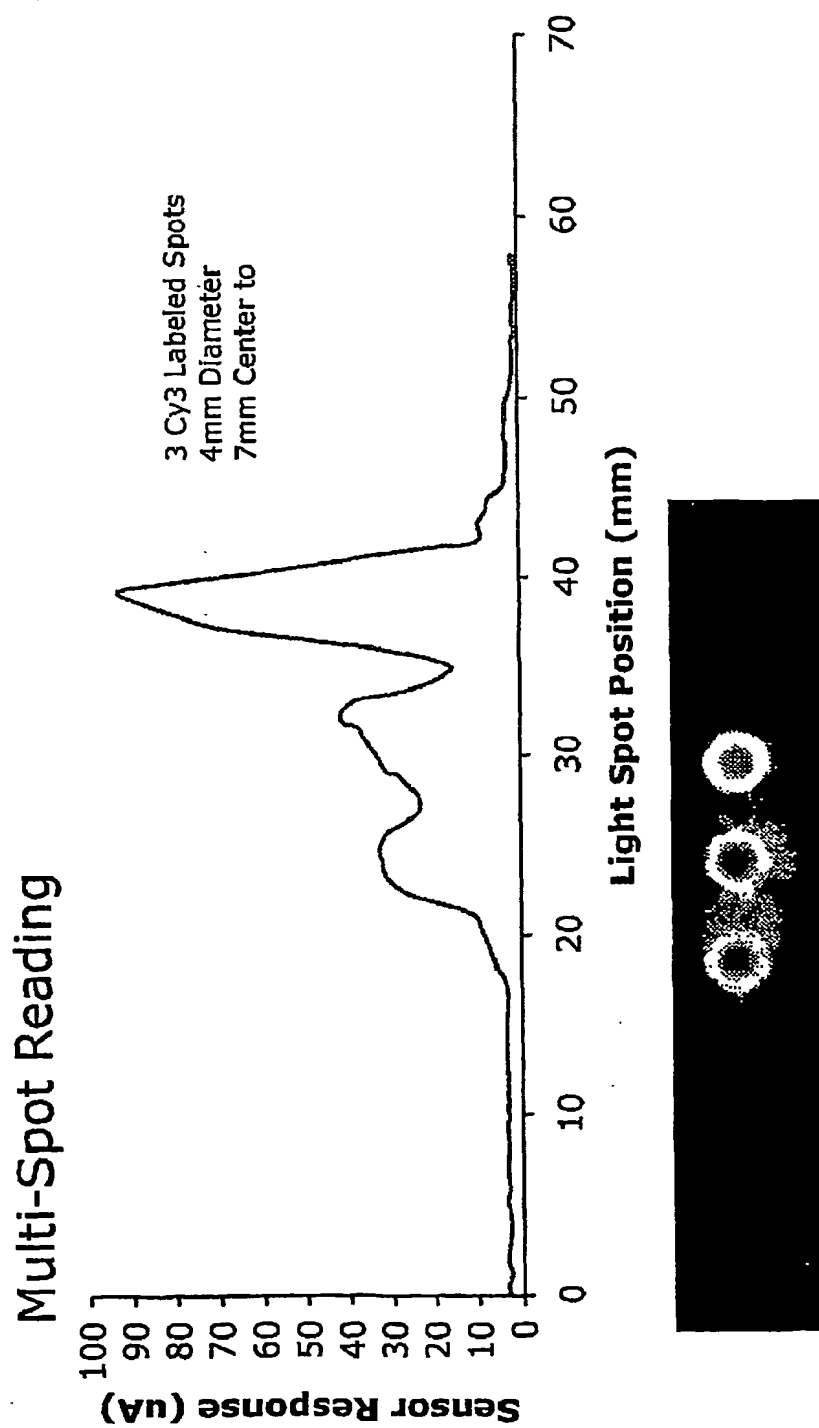
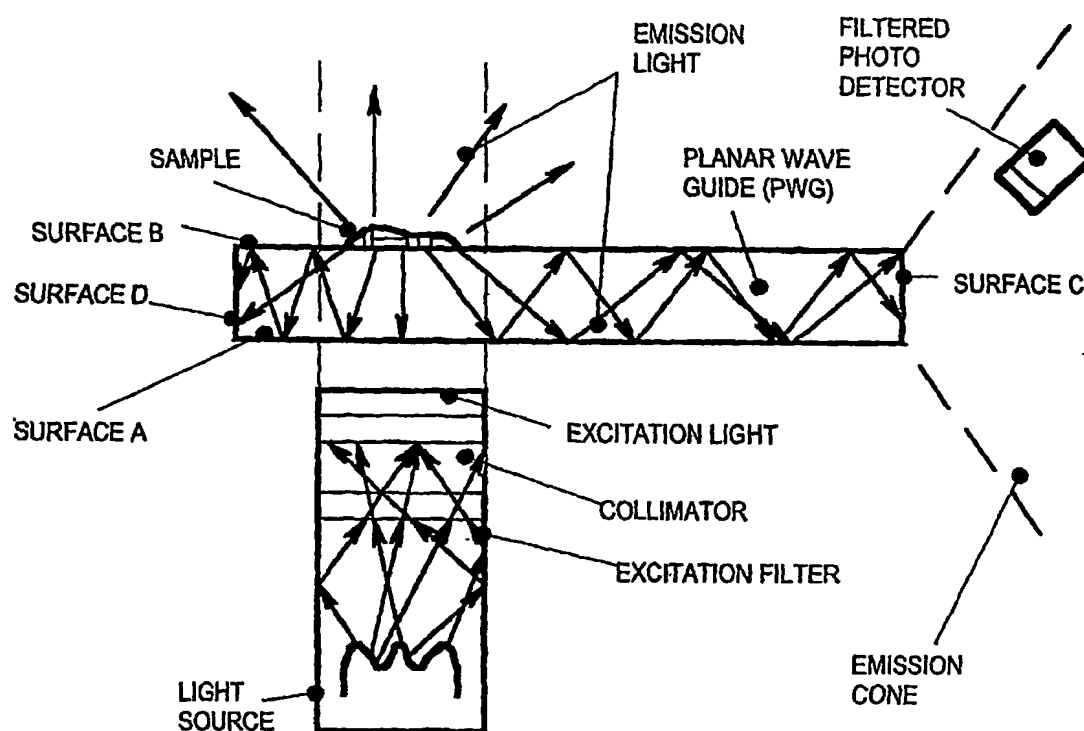
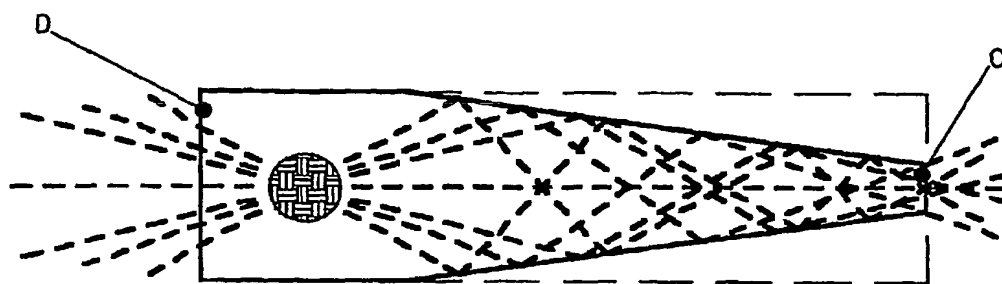


Figure 2



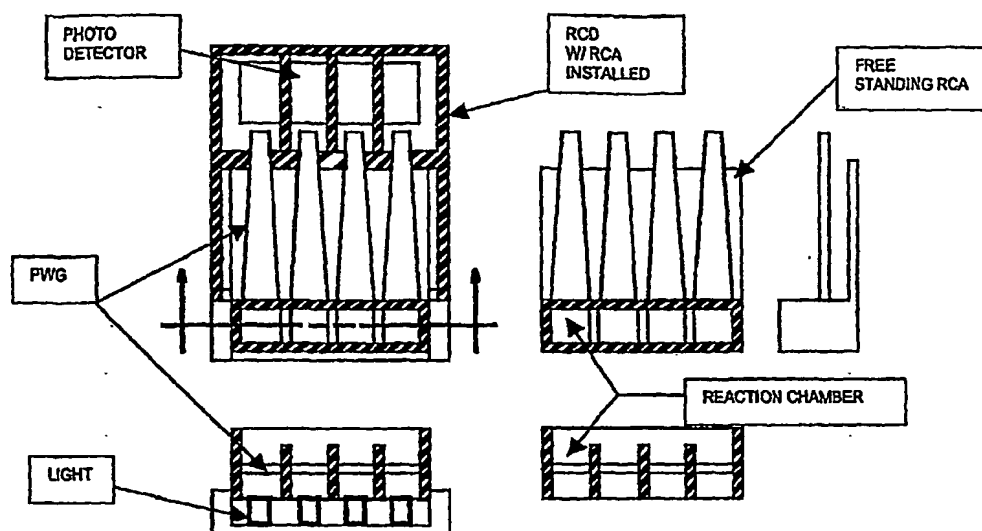
Light Path Illustration

Figure 3



Focusing PWG

Figure 4



Multiplexing Means 1 Illustration

Figure 5

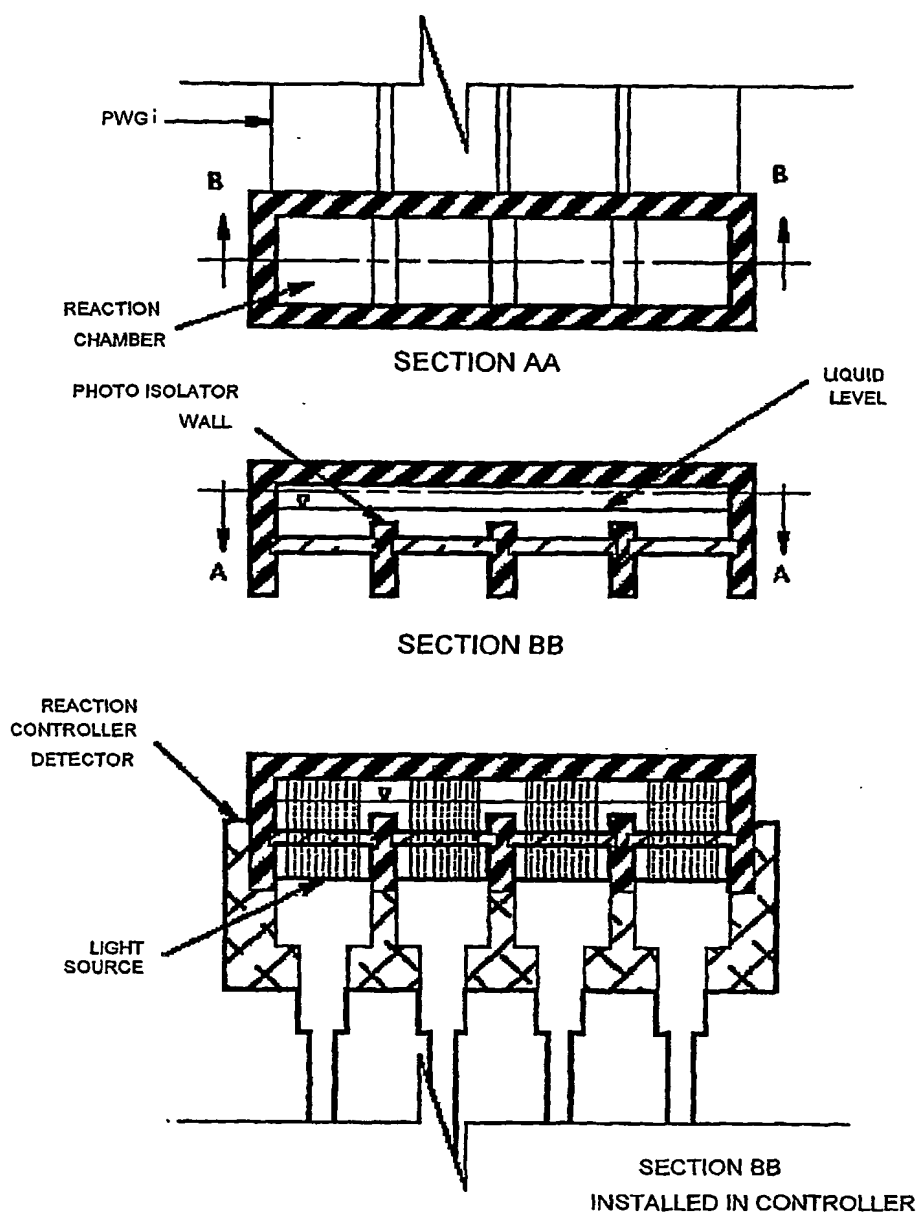
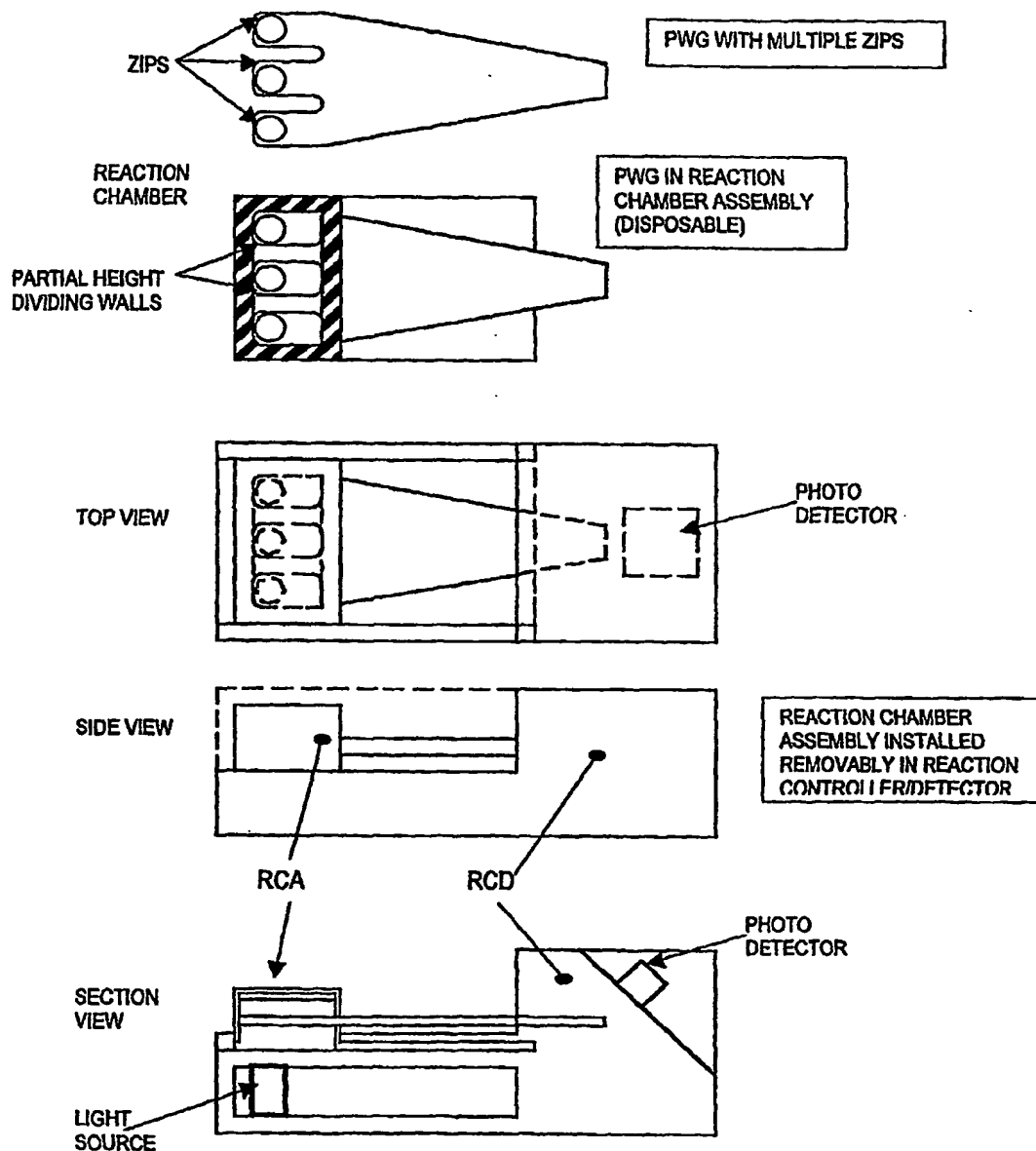


Photo Isolation Illustration

Figure 6



Multiplexing Means

Figure 7

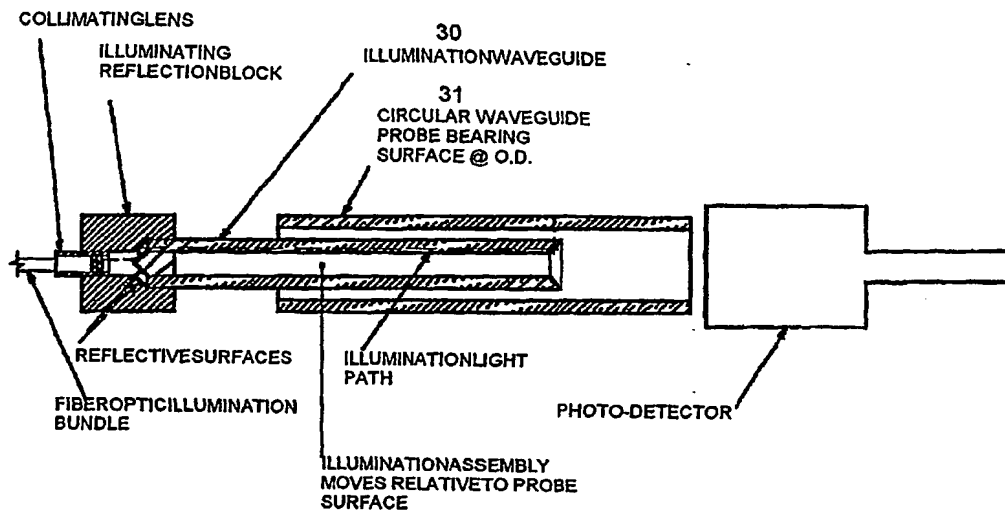
**Tubular CWG Detection**

Figure 8

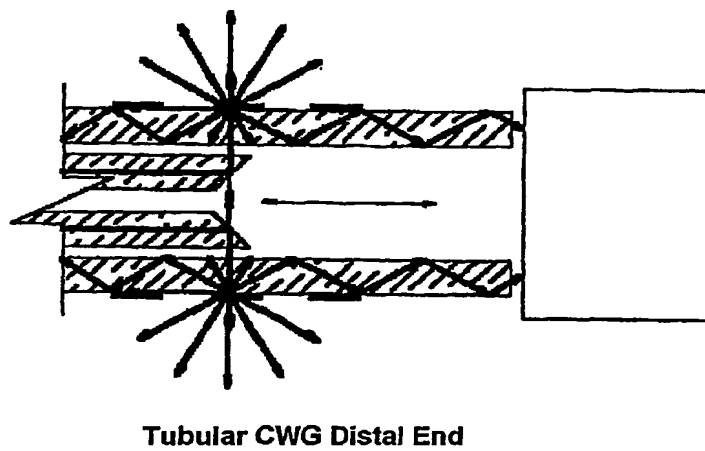
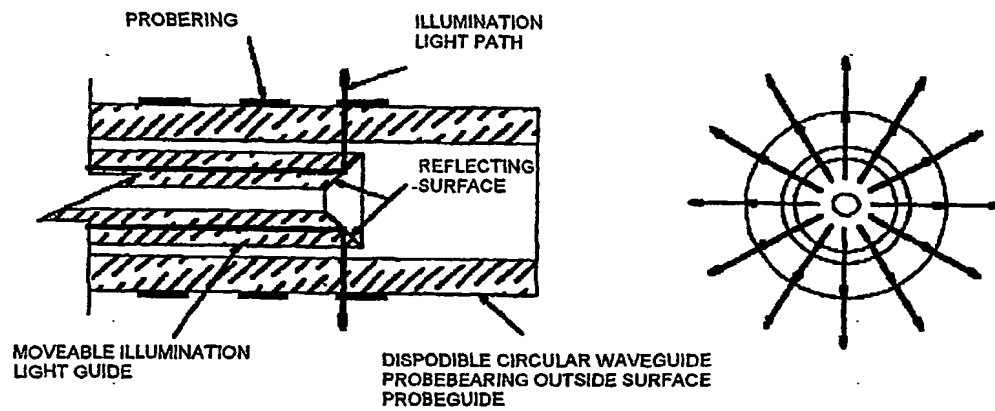
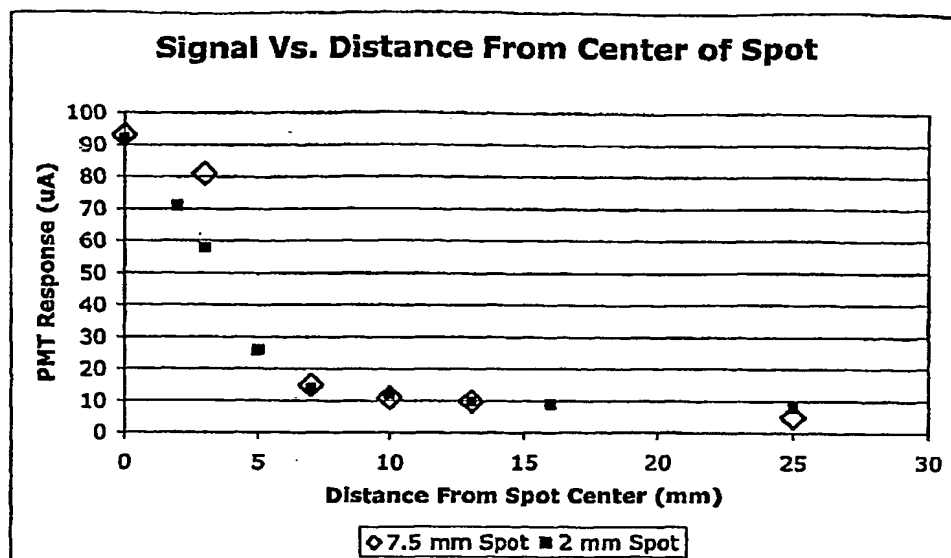
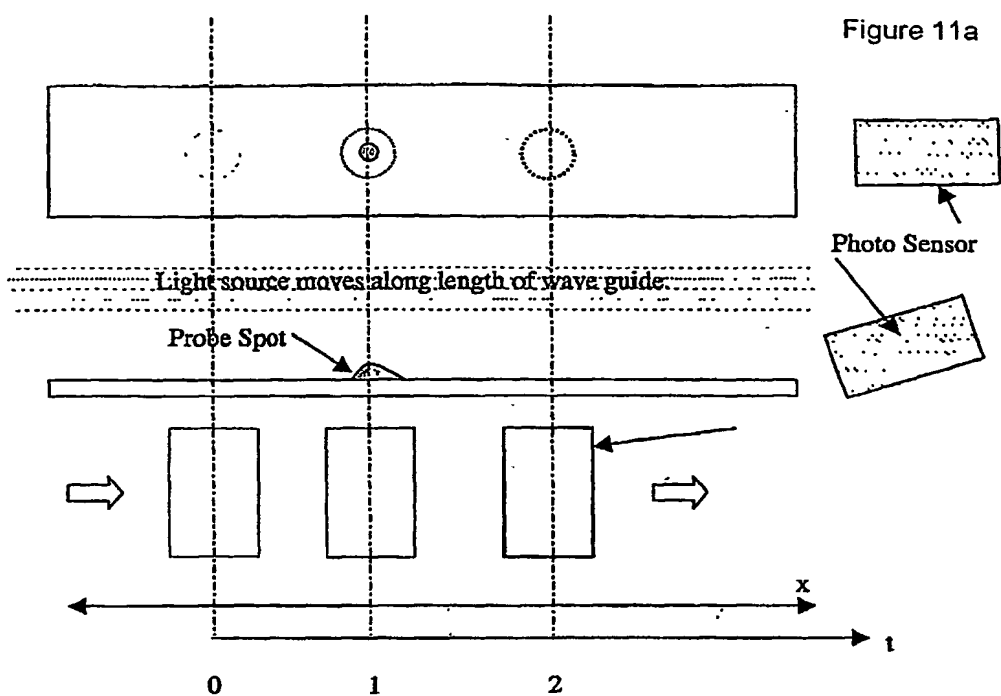


Figure 9



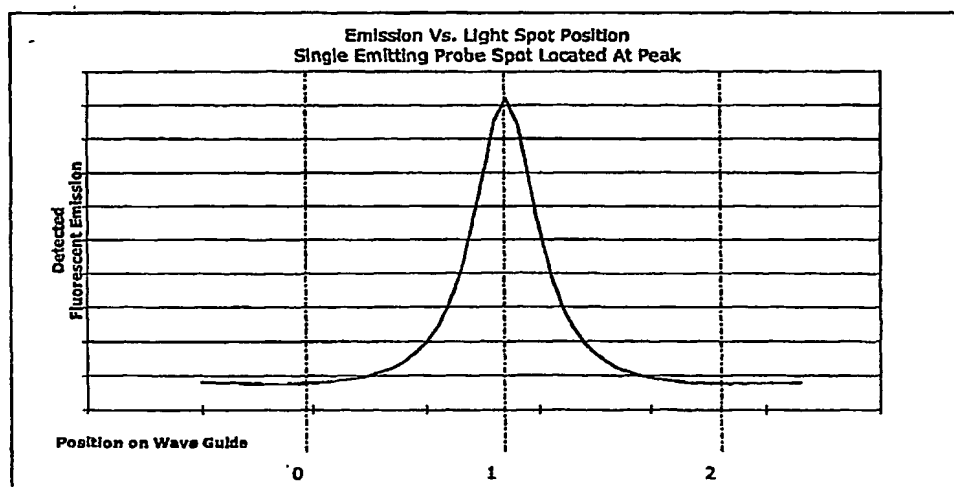
Emission Decay Data Vs. Excitation Light Alignment

Figure 10

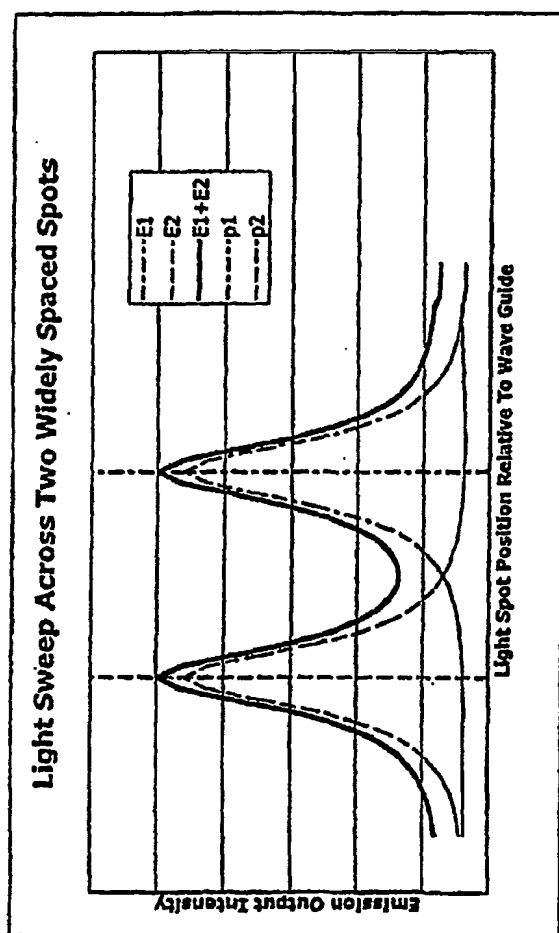


Data Collection:
simultaneous collection of light position and photo sensor output.

Figure 11b

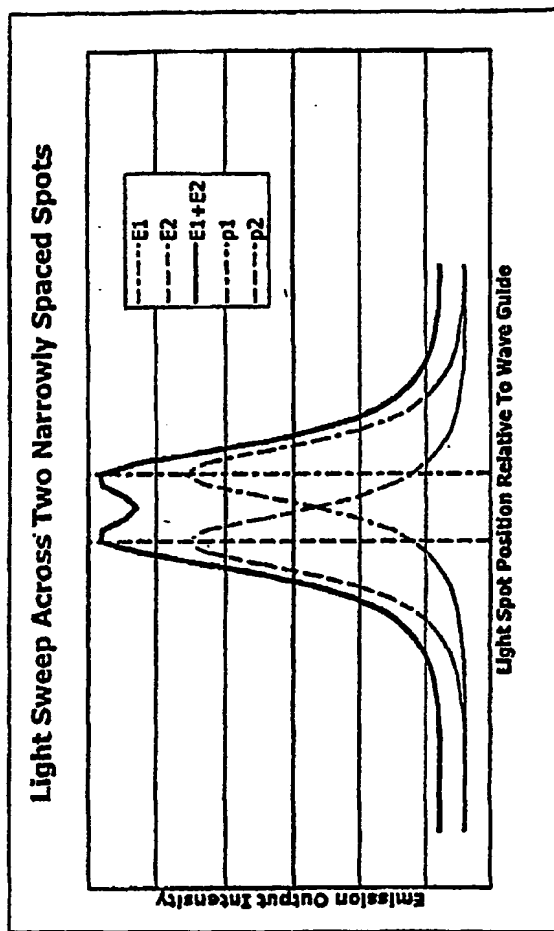


Data Collection Method & Projected Readings



Two Spot Sweep Data, Wide Spacing

Figure 12



Two Spot Sweep Data, Narrow Spacing

Figure 13

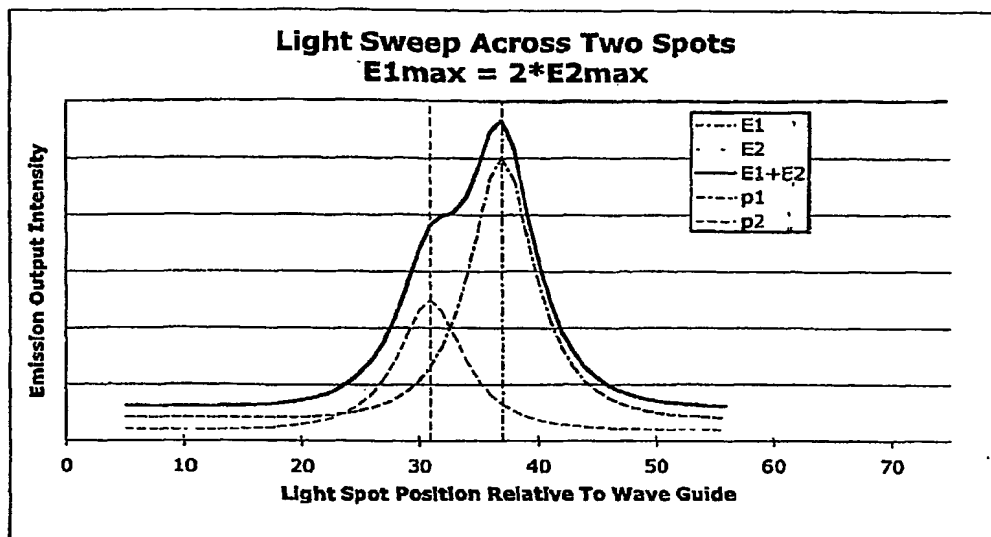


Figure 14

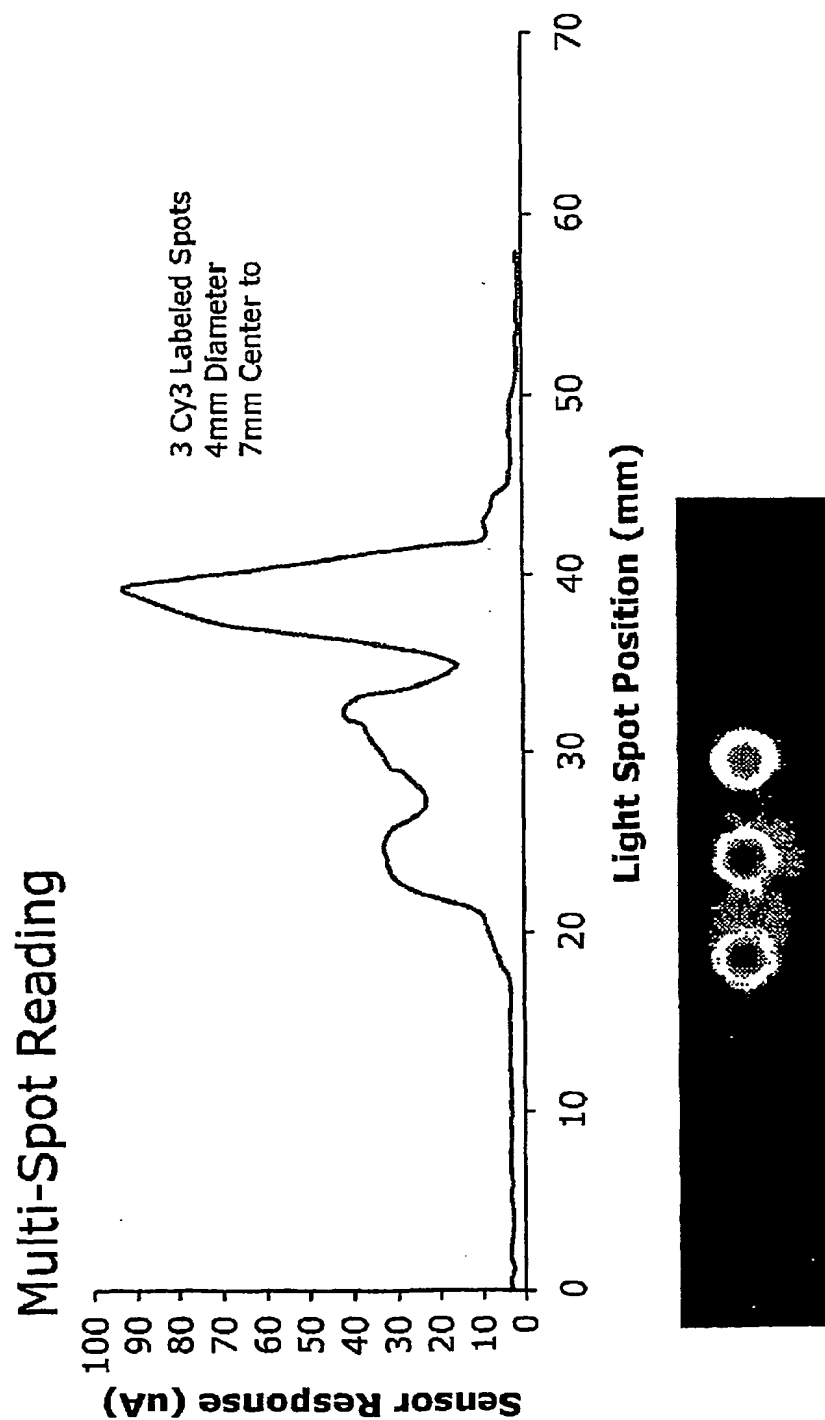


Figure 15

$$E = \sum_{i=1}^n E_i \quad (1)$$

- E: Total fluorescent emission output associated with a given excitation light spot location.
 E_i : Fluorescent emission generated from a fluorescent spot i .

Assumption 3 is based on empirical data that has been collected showing that such a relationship does exist. The derived relation, in its basic form is shown in equations 2 and 3.

$$E_i = E_{\max, i} \left\{ 1 - \left[\frac{(x_s - x_i)^2}{\beta + (x_s - x_i)^2} \right] + \left[\frac{\text{abs}(x_s - x_i)}{\alpha + \text{abs}(x_s - x_i)} \right] \right\} + Z \quad (2)$$

$$E_i = E_{\max, i}(A_{s,i}) + Z \quad (3)$$

- x_s : Position of excitation light source relative to waveguide surface coordinates.
 x_i : Position of fluorescent source location i .
 E_{\max} : Maximum fluorescent emission from source i . This emission occurs where $x_s = x_i$.
 β, α : Constants determined by dimensional parameters of slide, excitation light spot, and fluorescent source.
 Z : Background fluorescence measured.

This expression was derived from data taken of single spots hybridized to a slide (waveguide) surface.

Deconvolution Method

The actual mathematical method of deconvolution recognizes through assumptions 1, 2, and 3, that individual maxima of emission from each of the possible fluorescent sources can be determined by simultaneously solving a series of linear equations. The number of equations is equal to the number of unknown fluorescent source values that are sought. Following basic rules of linear algebra, the linear equations can be solved following the general expressions in Equation 4.

$$\begin{aligned} [A]\{E_{\max}\} &= \{E\} - Z \\ \{E_{\max}\} &= [A]^{-1}\{E\} - Z \end{aligned} \quad (4)$$

Figure.16

Deconvolution Compared to Collected Data

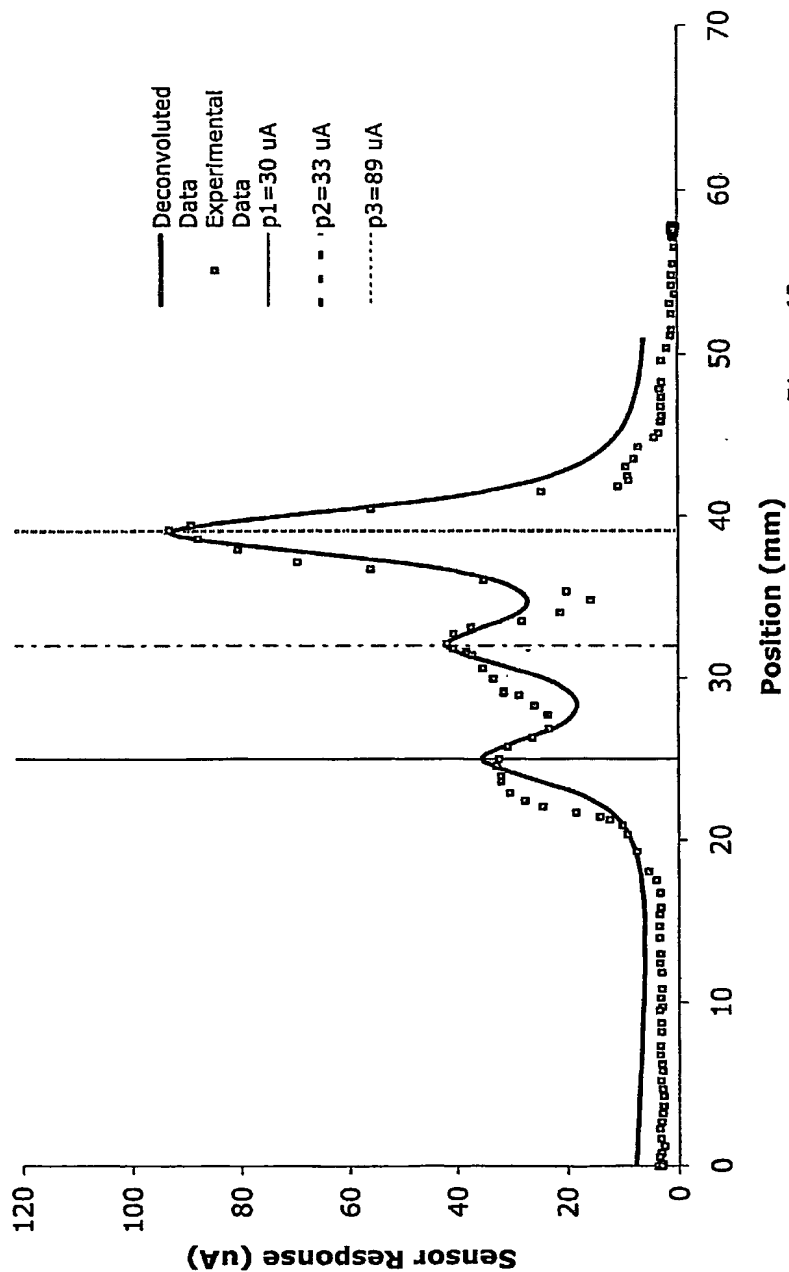


Figure 17

$$E_{\gamma} = \sum_{\gamma=1}^n E_{\gamma,\phi} + Z \quad (1)$$

E_{γ} : Total fluorescent emission output associated with a given excitation light spot location.

$E_{\gamma,\phi}$: Fluorescent emission generated from source ϕ .

$$E_{\gamma,\phi} = E_{\max,\phi} \left\{ 1 - \left[\frac{r_{\gamma,\phi}^2}{\beta + r_{\gamma,\phi}^2} \right] + \left[\frac{r_{\gamma,\phi}}{\alpha + r_{\gamma,\phi}} \right] \right\} \quad (2) \text{ \{Developed from empirical observations\}}$$

$$E_{\gamma,\phi} = E_{\max,\phi}(A_{\phi,\gamma}) + Z \quad (3)$$

$r_{\gamma,\phi}$: Distance between fluorescent source, ϕ , and light spot γ .

$E_{\max,\phi}$: Maximum fluorescent emission from source ϕ @ $r_{\gamma,\phi}=0$.

β, α : Constants determined by dimensional parameters of slide, excitation light spot, and fluorescent source.

Z : Background fluorescence measured.

$$\begin{aligned} [A]\{E_{\max}\} &= \{E\} - Z \\ \{E_{\max}\} &= [A]^{-1}\{E\} - Z \end{aligned} \quad (4)$$

The distance $r_{\gamma,\phi}$ as defined is derived from the vectors r_{γ} and r_{ϕ} as shown in Figure 19. The origin of the system shown in Figure 19 is predefined relative to the array. The method prescribes, through these assumptions and definitions, simultaneously solving a series of equations whose number is equal to that of the unknown loci (Equation 4). The product of deconvolution, $\{E_{\max}\}$, is the vector of individual and independent maxima of fluorescent emission from each potential source in the array.

Figure 18

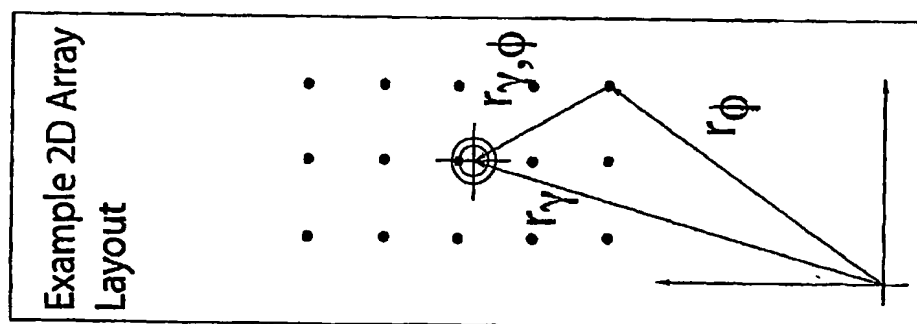


Figure 19

Homozygous Wild Type

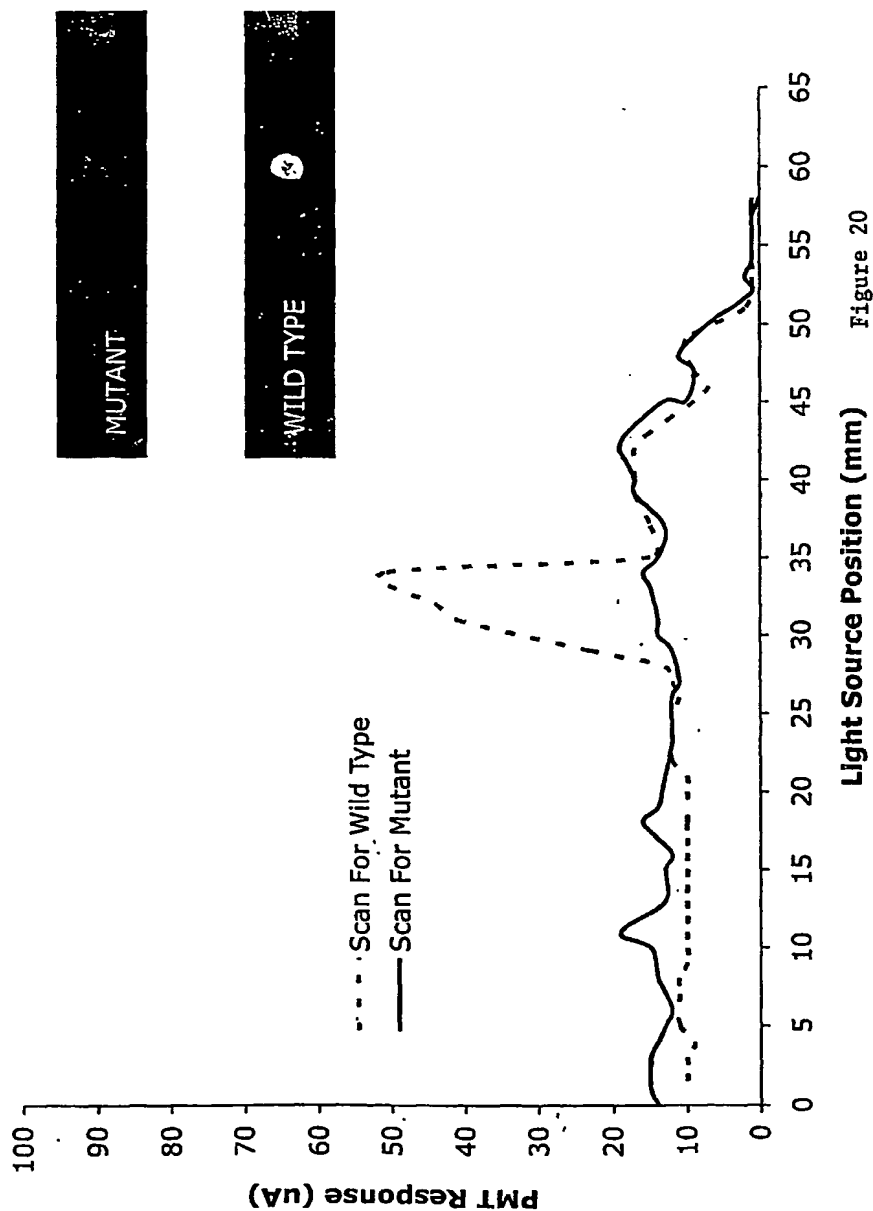


Figure 20

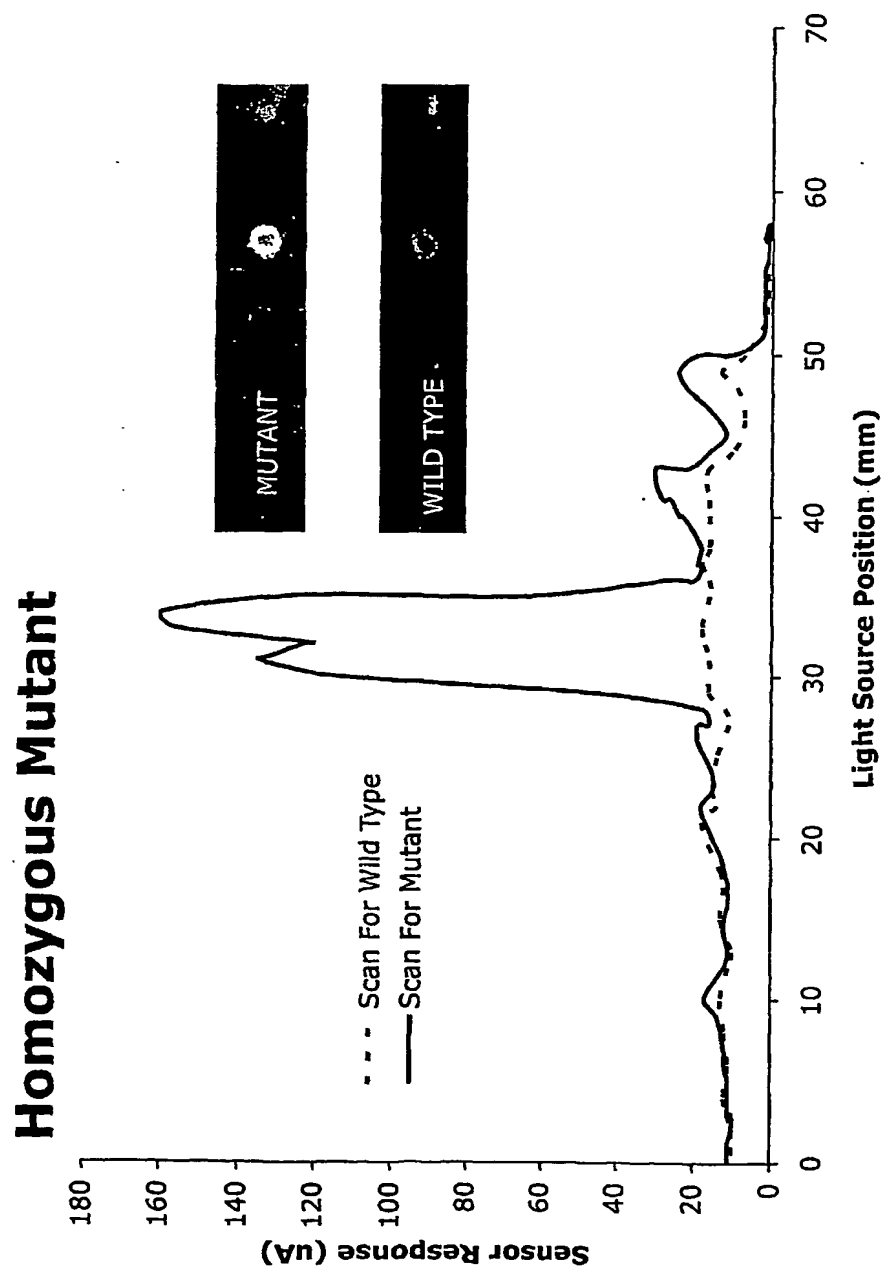


Figure 21

Spec. No.	Clinical		Laser Scanner		PWG Device	
	Wild Type	Mutant	Wild Type	Mutant	Wild Type	Mutant
1	+	-	+	-	+	-
2	+	-	+	-	+	-
3	+	-	+	-	+	-
4	+	-	+	-	+	-
5	+	-	+	-	+	-
6	+	-	+	-	+	-
7	+	-	+	-	+	-
8	+	-	+	-	+	-
9	+	-	+	-	+	-
10	+	-	+	-	+	-
11	+	-	+	-	+	-
12	+	-	+	-	+	-
13	+	-	+	-	+	-
14	+	-	+	-	+	-
15	+	-	+	-	+	-
16	+	-	+	-	+	-
17	+	-	+	-	+	-
18	-	+	-	+	-	+
19	-	+	-	+	-	+
20	-	+	-	+	-	+
21	-	+	-	+	-	+

Figure 22

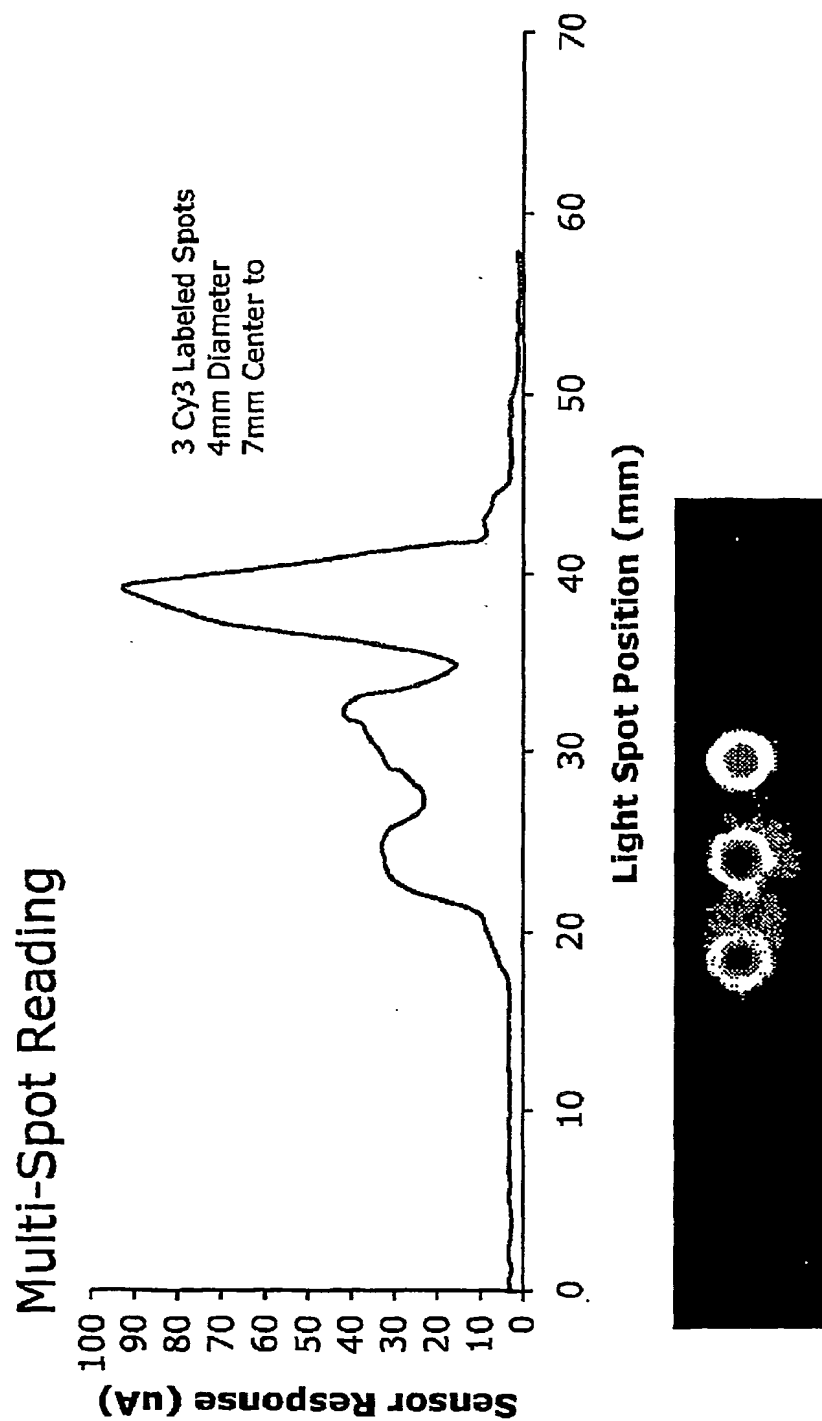


Figure 23

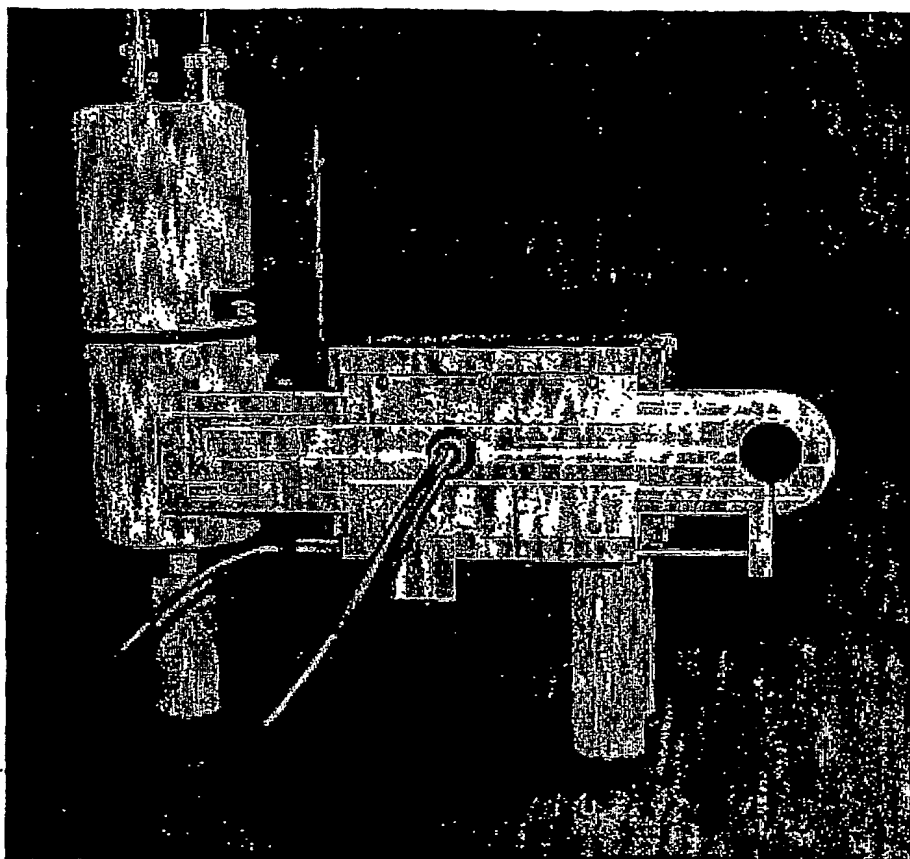


Figure 24

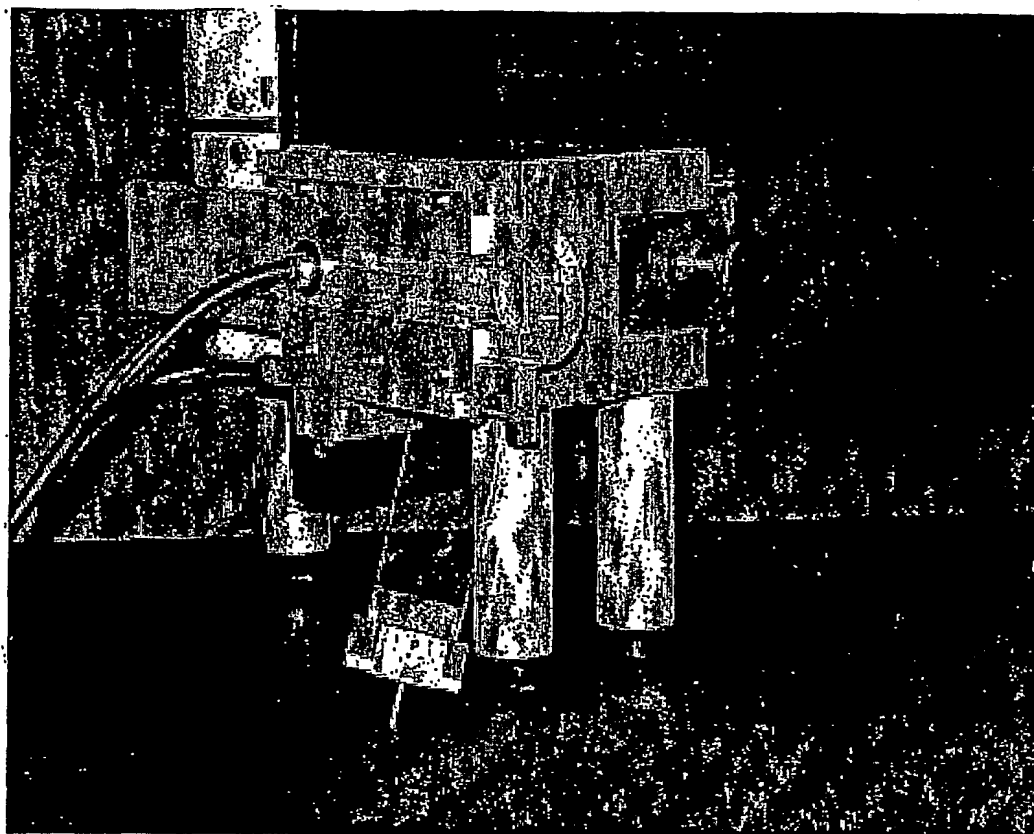
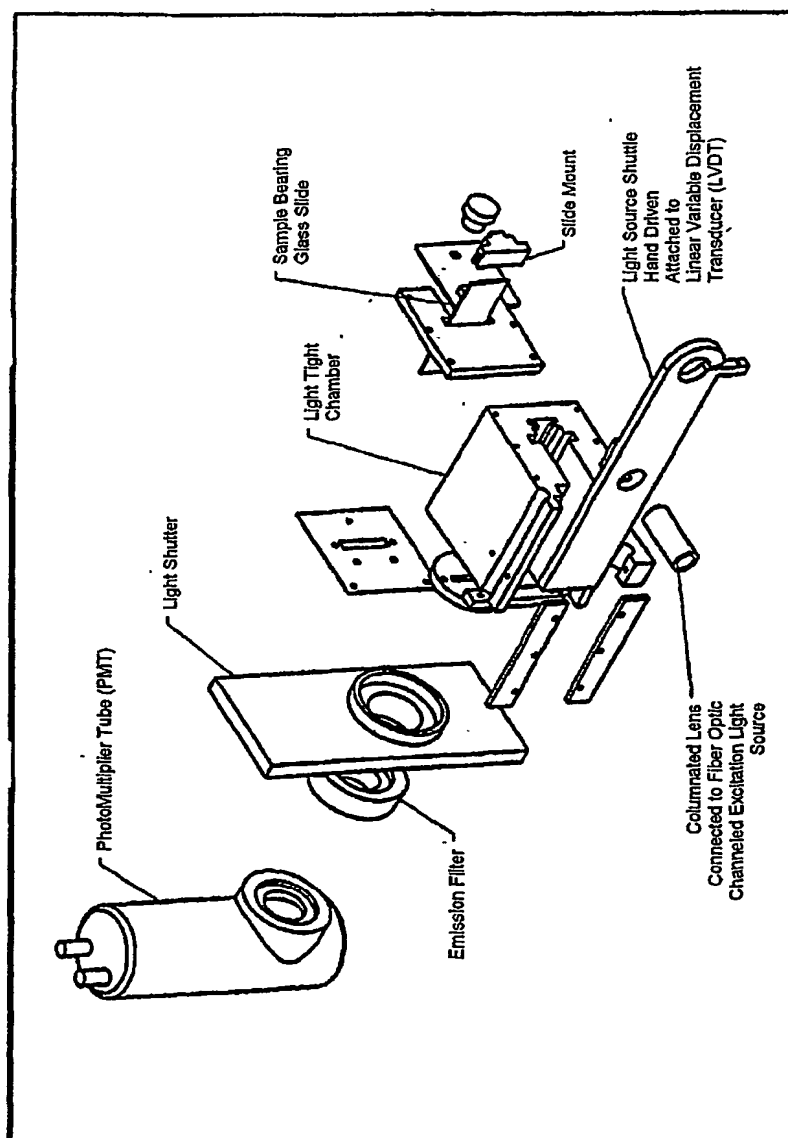


Figure 25



An Exploded Diagram of the System Depicted in the Photograph in Figure 25.

Figure 26